IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Moutsatsos et al..	Examiner:	Sandals W.
Serial No.:	09/148,234	Group Art Unit:	1636
Filed:	September 4, 1998		
Title:	GENETICALLY ENGINEERED CELLS WHICH EXPRESS BONE MORPHOGENIC PROTEINS		

DECLARATION UNDER RULE 37 C.F.R. 1.132

Assistant Commissioner for Patents
Washington, DC 20231

I, Dan Gazit, a citizen of Israel, residing at 46 Perez Berenstein Street, Jerusalem, 96920, hereby declare:

1. I am a Professor director the Biotechnology centre at the Hebrew University- of Jerusalem. I have a Ph.D. in bone biology from the Hebrew University, Jerusalem Israel. My fields of expertise are skeletal biotechnology and developmental molecular biology. Specifically I have been involved in the study of Adult Human Mesenchymal stem cells and Skeletal tissue engineering.

2. My Curriculum Vitae and list of publications are attached herewith as Appendix 1.

3. I have read the subject Application and have reviewed the patent Prosecution History, including the Office Action of June 15, 2004. The subject Application

describes *inter alia, ex-vivo* methods of transforming or transducing mesenchymal stem cells in vitro with a nucleic acid, which encodes for BMP-2 protein, for the implantation in a subject in need for bone repair or regeneration.

4. Claim 24 of the subject Application recites a method of inducing enhanced, organized, functional bone formation at a site of bone infirmity in a human, comprising the steps of:

   (a) transforming a cultured mesenchymal stem cell with a DNA encoding bone morphogenesis protein 2 (BMP-2);

   (b) culturing the cultured mesenchymal stem cell transformed in step (a), under conditions enabling expression of said DNA encoding bone morphogenesis protein 2; and

   (c) implanting said cultured mesenchymal stem cell at a site of bone infirmity

   whereby autocrine and paracrine effects of expressed bone morphogenesis protein 2 at said site of bone infirmity result in enhanced, organized, functional bone formation, thereby inducing functional bone formation at a site of bone infirmity.

5. In the Office Action, the Examiner rejected the claims of the above-identified Application as allegedly being obvious to one skilled in the art, based on Ahrens et al. (DNA and Cell Biology, Volume 12, NO. 10, pages 871-880, 1993) and in view of United States Patent No. 5,763,416 (Bonadio et al.) and United States Patent No. 6,048,964. The Examiner asserted that Bonadio allegedly discloses a method of producing cultured or bone marrow stromal cells for implantation at the site of bone infirmity by transforming the cells with recombinant bone morphogenetic protein. Specifically, the Examiner asserted "the cited references comprise teachings that provide a reasonable expectation of success in treating a site of bone infirmity in a human through the use of cultured mesenchymal stem cells that overexpress BMP-2".

6. The Examiner stated that Bonadio describes the use of bone progenitor cells transformed with a BMP for stimulating bone formation, and their functioning via autocrine and paracrine effects is expected. Further, the Examiner

contended that the motivation to combine the Bonadio and Ahrens references need only take into account a reasonable expectation of success in treating a site of bone infirmity in a human through the use of cultured mesenchymal stem cells that overexpress BMP-2, and the fact that Applicants data demonstrates the presence of autocrine and paracrine effects such cells demonstrates the fact that these mechanisms are necessarily present.

7. It is my opinion that the Examiner is incorrect in his assertion. Bonadio does not provide a credible foundation for a method of stimulating bone formation at a site of a bone infirmity by implanting a mesenchymal stem cell transformed/transduced with a BMP-2 construct. Though Bonadio describes that progenitor cells are targeted by his gene transfer methods, such a conclusion is not credible, in lieu of a direct demonstration by Bonadio, since much of the cell population targeted by direct gene transfer is not a stem or progenitor cell, which represents a small population of cells in vivo, at a site of bone infirmity. Moreover, uptake of the DNA by such cells *in situ*, is known to one skilled in the art to be drastically reduced (see for example, Rebel V.I. et al., Stem Cells (2000) 18: 176-82; Zhao Q. et al., Blood (1994) 84:3660-6), such that Bonadio does not credibly provide a foundation that BMP gene transfer provides more than paracrine effects for healing a bone infirmity.

8. Ahrens discloses *in vitro* responses of progenitor cells to a group of osteoinductive compounds (which include, *inter-alia,* a BMP), Ahrens provides no basis for the likelihood that implantation of such cells, transduced only with a vector expressing a BMP, *in vivo,* will stimulate bone induction at a site of bone infirmity. Such a result is predicated on appropriate cell homing and orientation along the defect edges, a result, which could not have been foreseen, based on Ahrens.

9. Further, Ahrens demonstrates differentiation of MSCs in vitro, which studies show (De Bari C. et al., Arthritis Rheum. 2004 Jan; 50(1):142-50) when implanted *in vivo*, these MSCs do not form functional tissue, and lose their cell surface marker phenotype. Thus, in view of the art cited, Ahrens does not credibly teach an ***exclusive*** effect of BMP-2, nor for that matter does Ahrens

credibly provide for an exclusive effect of any BMP, on mesenchymal stem cell bone induction. One skilled in the art would not believe the MSCs of Ahrens to be able to induce enhanced, organized, functional bone, once implanted in vivo. The combination of Ahrens with Bonadio do not credibly suggest that an ex-vivo cultured, BMP-2 transduced/transformed mesenchymal stem cell will form enhanced, organized, functional bone at a site of bone infirmity following implantation. Certainly both references do not unequivocally demonstrate an effect of BMP-2 alone, on MSCs for stimulating bone induction, nor suggest their role in stimulating, enhanced, organized, functional bone induction, specifically at a site of a bone infirmity.

10. Accordingly, it is my opinion that there is also no motivation to combine these references with a reasonable expectation of success for inducing enhanced, organized, functional bone formation at a site of bone infirmity in a human by implanting an ex-vivo cultured MSC transduced/transformed with any BMP, and in particular BMP-2. Both Bonadio and Ahrens disclosures do not produce a population of cells capable of forming organized, functional bone at a site of bone infirmity, the former, due to the improbability of obtaining such a cell, and the latter, due to the improbability of obtaining a cell that would function *in situ*, and the fact that there isno osteoinductive compound functioning alone defined.

11. The combination of Bonadio and Ahrens could not possibly have predicted the unexpected results obtained in the claimed invention, which resulted in enhanced, organized, functional bone formation at a site of bone infirmity. In vivo studies (*Gazit et al., 1999, J Gene Med 1: 121-133,* a copy of which is attached hereto as Appendix 3), demonstrated that engineered progenitor cells (C3H-BMP2), in comparison to administration of 3 μg recombinant human BMP2, or engineered non progenitor cells (CHO-BMP2) produced enhanced bone formation, and most surprisingly, that the formation was in alignment with the original defect edge, this despite the fact that greater amounts of BMP-2 were secreted from the CHO BMP-2 cells.

12. Contrary to the Examiner's assertion, these studies are very apropos to the disclosures of Ahrens and Bonadio, which at best credibly describe paracrine effects of BMP-2, as neither Bonadio nor Ahrens credibly describe targeting of progenitor cells *in situ* or lone effects of any BMP, in particular BMP-2. Thus, neither Ahrens, nor further in view of Bonadio credibly describe a means of providing the enhanced, organized, functional bone at a site of bone infirmity, as claimed in the instant invention.

13. In view of the reasons and the facts described above, one skilled in the art would not be able to predict the enhanced, organized, functional bone induction at a site of bone infirmity produced via implantation of *ex vivo* transformed/transduced MSCs with BMP-2, as claimed in the subject Application.

The undersigned further declares that all statements made herein of his own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 9/19/04 _

Dan Gazit

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BIOGRAPHICAL SKETCH			
NAME Gazit Dan	POSITION TITLE Associate Professor		
EDUCATION/TRAINING	DEGREE	YEAR	FIELD OF STUDY
Hebrew University of Jerusalem	D.M.D.	1970-1976	Dental Surgeon
Hebrew University of Jerusalem	Ph.D.	1986-1991	Bone Biology

Professional Experience

1981-1985	Instructor in Oral Pathology, Oral Pathology, Hebrew University of Jerusalem
1986-1992	Lecturer in Oral Pathology, Bone Biology and Pathology, Hebrew University of Jerusalem
1990-1992	Visiting Professor, Bone Biology, UCSF
1992-1999	Tenured Senior Lecturer in Oral Pathology, Bone Biology, Biotechnology and Pathology, Hebrew University of Jerusalem
1996-2001	Director, Oral Pathology, Oral Pathology Biopsy Facility, The Hebrew University-Hadassah Faculty of Dental Medicine
1997-	Director, Hebrew University Dental Sciences Graduate Program
1999	Visiting Professor, Bone Biology, Leiden Medical Center
1999	Visiting Professor, Bone Biology, Boston University
1999	Visiting Professor, Bone Biology, Harvard Medical School
1999-	Associate Professor, Bone Biology and Biotechnology, Hebrew University of Jerusalem

Professional Membership

1999-	Gene Therapy Steering Committee Member, Gene Therapy Center, Hadassah Medical Center, Jerusalem
2002-	Chairman of Biotech Committee, Hebrew University of Jerusalem

Selected peer-reviewed publications

1. **Gazit, D.**, Zilberman, Y., Ebner, R. and Kahn, A. (1998) Evidence that bone loss (osteopenia) in old, male mice results from the diminished activity and availability of TGF-β1. J. Cell. Biochem. 70:478-488.

2. **Gazit, D.**, Zilberman, Y., Turgeman, G., Zhou, S., and Kahn A. (1999) Recombinant TGF-β1stimulates bone marrow osteoprogenitor cell activity and bone matrix synthesis in osteopenic, old male mice. J. Cell. Biochem. 73:379-389.

3. **Gazit, D.**, Turgeman, G., Kelley, P., Wang, E., Jalenak, M., Zilberman, Y. and I.K. Moutsatsos. (1999). Engineered pluripotent mesenchymal cells integrate and differentiate in regenerating bone: A novel cell-mediated gene therapy. J Gene Med. 1:121-133.

4. Moutsatsos IK, Turgeman G, Zhou S, Kurkalli BG, Pelled G, Tzur L, Kelley P, Stumm N, Mi S, Muller R, Zilberman Y, **Gazit D.** (2001) Exogenously regulated stem cell-mediated gene therapy for bone regeneration. Mol Ther. 3(4): 449-61.

5. Turgeman G, Pittman DD, Muller R, Kurkalli BG, Zhou S, Pelled G, Peyser A, Zilberman Y, Moutsatsos IK, **Gazit D.** (2001) Engineered human mesenchymal stem cells: a novel platform for skeletal cell mediated gene therapy. J Gene Med. 3(3): 240-51.

6. Zhou S, Zilberman Y, Wassermann K, Bain SD, Sadovsky Y, **Gazit D.** (2001) Estrogen modulates estrogen receptor α and β expression, osteogenic activity, and apoptosis in mesenchymal stem cells (MSCs) of osteoporotic mice. J Cell Biochem. Suppl. 36:144-55.

7. Honigman A, Zeira E, Ohana P, Abramovitz R, Tavor E, Bar I, Zilberman Y, Rabinovsky R, **Gazit D**, Joseph A, Panet A, Shai E, Palmon A, Laster M, Galun E. (2001) Imaging transgene expression in live animals. Mol Ther. 4(3): 239-49.

8. Alexander JM, Bab I, Fish S, Muller R, Uchiyama T, Gronowicz G, Nahounou M, Zhao Q, White DW, Chorev M, **Gazit D**, Rosenblatt M. (2001) Human parathyroid hormone 1-34 reverses bone loss in ovariectomized mice. J Bone Miner Res 16(9): 1665-73.

9. Hoffmann A, Czichos S, Kaps C, Bachner D, Mayer H, Zilberman Y, Turgeman G, Pelled G, Gross G, and **Gazit D.** (2002) The T-Box transcription factor Brachyury mediates cartilage development in mesenchymal stem cell line C3H10T1/2. J. Cell Science. 115, 769-781.

10. Turgeman G, Zilberman Y, Zhou S, Kelly P, Moutsatsos I.K, Kharode YP., Borella LE., Bex FJ., Komm BS., Bodine PVN., and **Gazit D** (2002). Systemically administrated rhBMP-2 promotes

MSC activity and reverses bone and cartilage loss in osteopenic mice. J. Cell Biochem, 86(3):461-474.

11. Pelled G, Turgeman G, Aslan H, Gazit Z, and **Gazit D.** (2002) Mesenchymal stem cells for bone gene therapy and tissue engineering. Current Pharmaceutical Design, 8; 99-110.

12. Zilberman Y, Turgeman G, Pelled G, Xu N, Moutsatsos IK, Hortelano G, and **Gazit D** (2002). Polymer encapsulated engineered mesenchymal stem cells secrete exogenously regulated rhBMP-2, and induce osteogenic and angiogenic tissue formation. PAT, 13; 863-870.

13. Zhou S., Turgeman G., Harris SE.,Leitman DC., Komm BS., Bodine PVN., and **Gazit D** (2003). Regulation of murine BMP-2 gene transcription by ERα and β in mesenchymal stem cells. Mol. Endocrinol. 17(1):56-66.

14. Turgeman G., Aslan H., Gazit Z., and **Gazit D.** (2002). Cell mediated gene therapy for bone formation and regeneration. Current Opinion in Molecular Therapeutics. 4(4):390-4

15. Ehrenfreund-Kleinman T., Gazit Z., **Gazit D.**, Azzam T., Golenser J. and Domb AJ. (2002) Synthesis and biodegradation of Arabinogalactan sponges prepared by reductive amination. Biomaterials, 23(23): 4621-4631.

16. Bar I., Zilberman Y., Turgeman G., Zeira E., Galun E., Honigman A., Turgeman G., Clemens T., Gazit Z., **Gazit D.** Molecular Imaging of the skeleton: quantitative real time bioluminescence in transgenic mice. J Bone Miner Res, In press, 2003.

17. Gafni Y., Gazit Z., **Gazit D.** Stem cells as vehicles for orthopedic gene therapy. Gene therapy, Accepted for publication, 2003.

18. Aslan,H.; Zhou, S.; Pelled,G.; Turgeman, G.; Mclarney, S.; Komm, B.; Bodine, P.; **Gazit, D.** Transcriptional profiling of estrogen-induced osteogenic differentiation of Murine adult mesenchymal stem cells (AMSCs) in vitro, chapter 11 in: Mesenchymal Stem cells: Biology and Potential Clinical Uses (Grisolía, S.; Minyana, D. & Bendala-Tufanisco, E., eds.) Ministerio de Sanidad y Consumo, Madrid, Spain. In press, 2003.